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Targeting the ERK1/2 and ERK5 pathways simultaneously induces mesenchymal to epithelial transition in TNBC

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Introduction

- Extracellular signal-regulated kinase (ERK) 5, a member of mitogen activated protein kinase (MAPK) family, is an emerging target in cancer therapeutics. Activation of ERK5 via overexpression induces EMT and hormone-independent growth of breast cancer. EMT leads to the loss of cell polarity, downregulation of E-cadherin, and upregulation of mesenchymal markers snail, zinc-finger E-box binding homeobox (ZEB1), and vimentin. EMT is also associated with drug resistance. Although ERK1/2 and ERK5 activation is known to mediate EMT, the effect of ERK1/2 and ERK5 inhibition on mesenchymal to epithelial transition (MET), the reverse of EMT, is poorly understood in cancer.

- Triple negative breast cancer (TNBC) cells have a mesenchymal phenotype and show poor sensitivity to chemotherapy agents. The loss of ER, PR, and HER2 contributes to the aggressive state of the disease and lack of targeted therapy. Activation of the intracellular signaling pathways such as the MAPK pathway mediates tumorigenesis in TNBCs.

- In the present study, the effect of dual ERK1/2 and ERK5 inhibition on MET, cell viability, migration, and anchorage-independent growth was evaluated in TNBC. ERK1/2 and ERK5 activities were modulated via pharmacological inhibitors and molecular tools. Cell morphology and protein expression of EMT markers E-cadherin and ZEB1 were evaluated. XMD8-92, an ERK5 inhibitor was found to synergize with doxorubicin in lung cancer. Therefore, the effect of ERK1/2 and ERK5 inhibition on doxorubicin sensitivity was evaluated.

Hypothesis

MAPK inhibition will induce MET and sensitize triple negative breast cancer cells to doxorubicin.

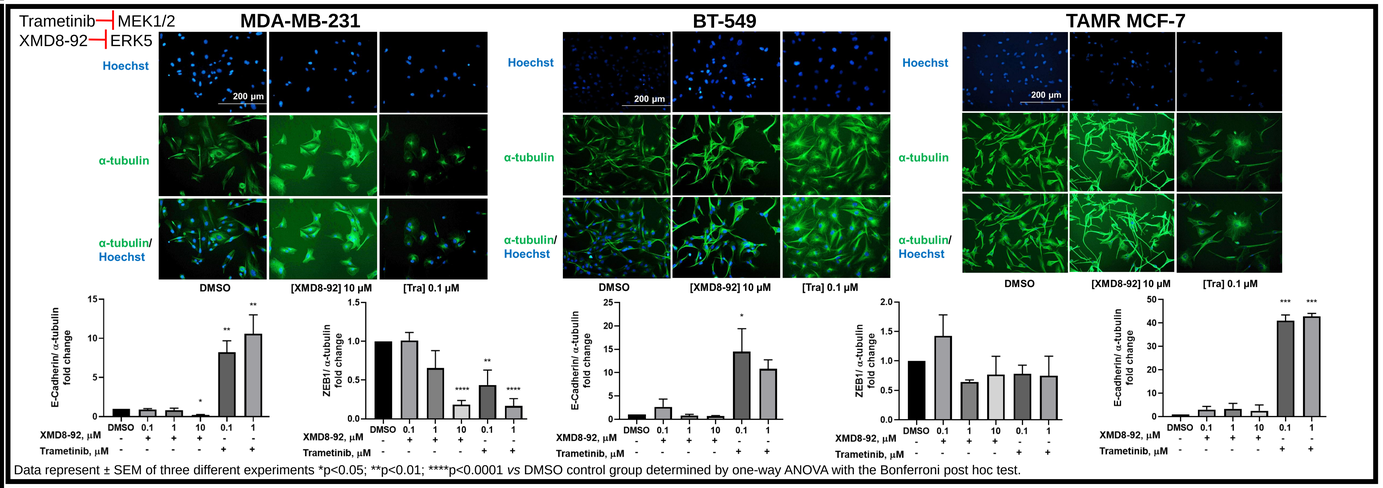
Methods

- **Cell Culture:** MDA-MB-231, BT-549, and MCF-7 cells were obtained from ATCC. Cells were cultured as directed by the manufacturer guidelines.

- **Western Blot:** 30 µg of protein was loaded on a 8% SDS-PAGE and transferred to a nitrocellulose membrane. Membranes were incubated with primary (CST) and Goat anti-rabbit 680 LT and Goat anti-mouse 800 CW secondary antibodies. Membranes were scanned on an Odyssey Infrared Imager (LI-COR Biosciences) and analyzed using Image Studio Lite Software.

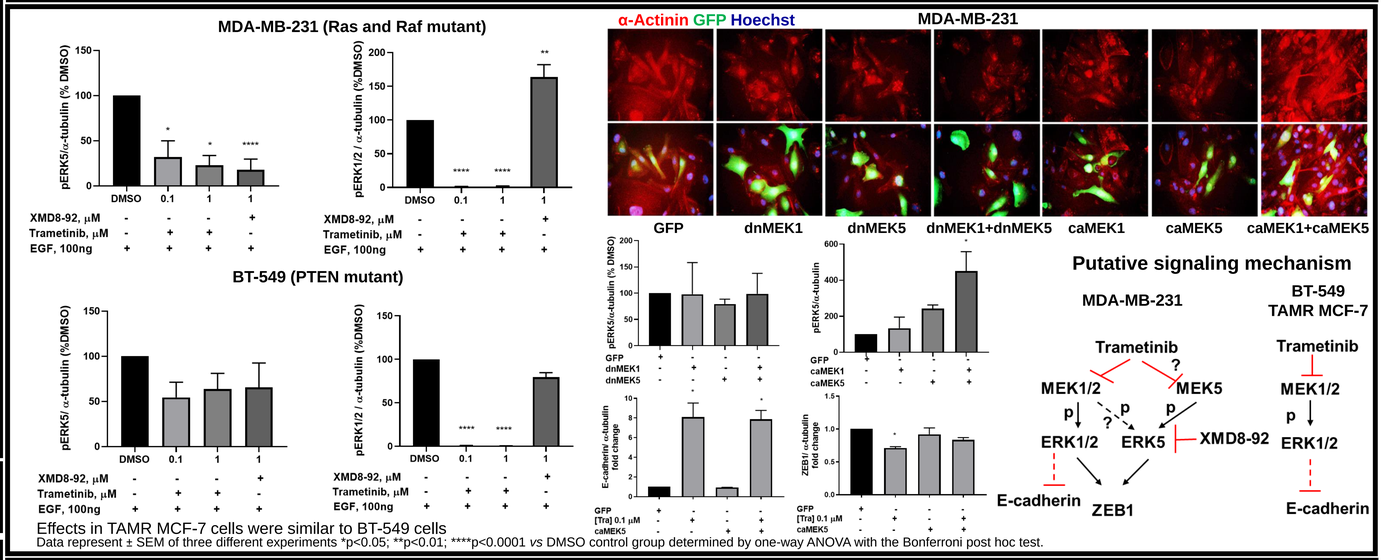
- **MTT Assay:** MDA-MB-231 cells were plated at a density of $5x10^3$ cells per well in 96 well plates. MTT was added to each well and were incubated for 3 hours. Absorbance was measured at 570 nm using VICTOR3 1420 multi-label counter, Perkin Elmer).

Pharmacological inhibition of ERK5 and ERK1/2 induces MET in breast cancer



Data represent ± SEM of three different experiments *p<0.05; **p<0.01; ****p<0.0001 *vs* DMSO control group determined by one-way ANOVA with the Bonferroni post hoc test.

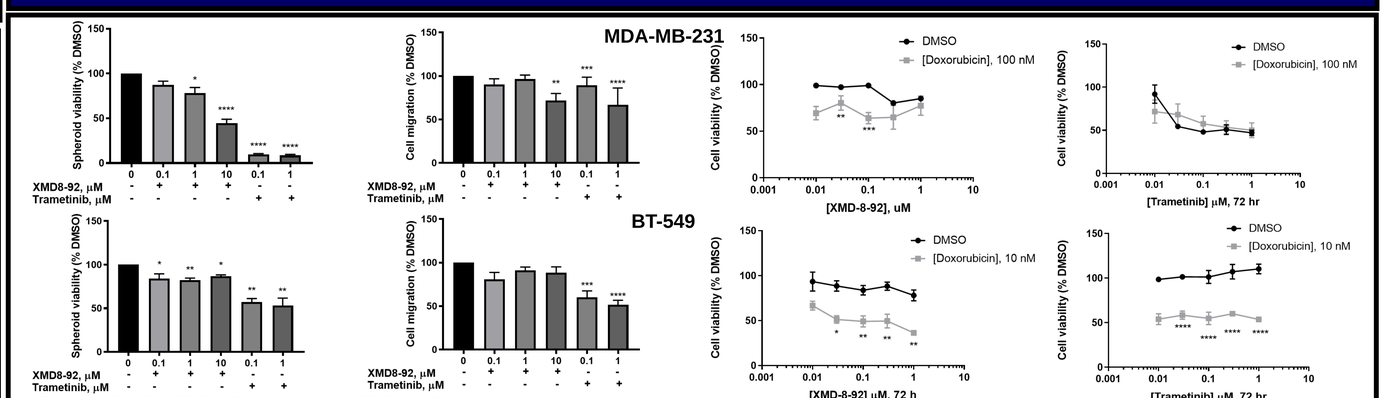
MEK1/2 and MEK5 mediate ERK5 activation and EMT in MDA-MB-231 cells



Effects in TAMR MCF-7 cells were similar to BT-549 cells

Data represent ± SEM of three different experiments *p<0.05; **p<0.01; ****p<0.0001 *vs* DMSO control group determined by one-way ANOVA with the Bonferroni post hoc test.

ERK5 and ERK1/2 inhibition decreases cell migration, spheroid formation, and enhances doxorubicin sensitivity in TNBC cells



Effects in TAMR MCF-7 cells were similar to BT-549 cells

Data represent ± SEM of three independent experiments *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001 *vs* DMSO control group determined by one-way ANOVA and two-way ANOVA with the Bonferroni post hoc test.

Summary, Conclusions, and Future Directions

Summary

- ERK1/2 and ERK5 inhibition induces MET in MDA-MB-231 cells.
- XMD8-92 induces MET in MDA-MB-231 cells via downregulation of ZEB1.
- Trametinib induces MET via inhibition of ERK1/2-RSK signaling in BT-549 and TAMR-MCF-7 cells.
- Trametinib and XMD8-92 decrease spheroid formation and cell migration in TNBC cells.

Conclusion

- E-cadherin may be an effective biomarker to predict response to MEK1/2 pathway inhibition.
- Constitutive activation of the AKT pathway in BT-549 cells may be the reason for reduced efficacy of kinase inhibitors on MET.

Future directions

- Evaluate mechanism of synergy between kinase inhibitors and doxorubicin.
- Evaluate the effect of MEK1/2 and MEK5 pathway inhibitors in combination with CDK4/6 inhibitors on cell proliferation.

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